Cloning and Expression of a Prokaryotic Enzyme, Arginine Deiminase, from a Primitive Eukaryote * Giardia intestinalis*

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Arginine deiminase (EC 3.5.3.6) catalyzes the irreversible catabolism of arginine to citrulline in the arginine dihydrolase pathway. This pathway has been regarded as restricted to prokaryotic organisms but is an important source of energy to the primitive protozoan *Giardia intestinalis*. In this paper we report the cloning and expression of the arginine deiminase gene from this parasite. Degenerate oligonucleotides based on amino acid sequences of tryptic peptides from the purified protein were used to amplify a portion of the arginine deiminase gene. This was then used as a probe to screen *HindII* and *PstI* “mini” libraries to obtain two overlapping clones that contained the arginine deiminase gene. The open reading frame encoded 581 amino acids including all of the tryptic peptides that were sequenced and corresponded to a molecular mass of 67 kDa. Northern blot analysis identified a single 1.8-kilobase transcript in both trophozoites and encysting cells. Arginine deiminase was successfully expressed in *Escherichia coli* and purified to homogeneity. The recombinant protein was found to have characteristics comparable with those of the native enzyme.

*Giardia intestinalis* is one of the most commonly transmitted intestinal pathogens in the world. There are two stages in the life cycle, both well adapted to two different, hostile environments. Infection is most often by ingestion of the cyst from a contaminated water or food supply. Exycystation is induced by passage through the stomach, resulting in the emergence of the motile trophozoite form, which colonizes the upper small intestine and causes the symptoms of giardiasis, including vomiting and diarrhea, by an as yet unknown mechanism. Completion of the life cycle occurs when trophozoites are carried downstream by the intestinal fluid and then encyst, followed by emergence from the host. Current treatment against giardial infection is problematic, due to the increasing emergence of drug-resistant strains and lack of compliance. Hence there is a need for new and specific drugs against infection.

In addition to being a major health burden, *G. intestinalis* has recently been described as the “missing link” between prokaryotes and eukaryotes (1). Therefore *Giardia* is an important tool for the study of the evolution of the complex nature of the eukaryotic cell. Metabolism in *Giardia*, for example, is indicative of the primitive nature of this organism. In *Giardia*, the arginine dihydrolase (ADH) pathway is a major energy source, with arginine the preferential fuel used in the early and most proliferative stages of *in vitro* growth (2). This pathway has been reported in only two other eukaryotes, *Trichomonas vaginalis* (3) and *Trichomonas fetus* (4), both also placed on early branches of the eukaryotic evolutionary tree (5).

The ADH pathway is normally confined to the prokaryotic kingdom, where it provides both a source of energy and nitrogen (6). The pathway consists of three enzymatic steps, involving arginine deiminase, ornithine transcarbamoylase, and carbamate kinase, with ATP production at the final enzymatic step as follows: arginine → citrulline → ornithine + carbamyl phosphate → CO₂ + NH₄⁺ + ATP.

The activities of all the arginine dihydrolase pathway enzymes have been demonstrated in *Giardia* (7), with carbamate kinase being the most active enzyme yet reported in cell-free extracts. The activity of ornithine transcarbamylase, the least active enzyme in the pathway, is approximately 5-fold higher than the reported activities of some glycolytic enzymes (7, 8). An arginine-ornithine exchange transporter has been described in *Giardia* that has properties similar to arginine transporters found in some prokaryotes that utilize the ADH pathway (9).

The function of the pathway in intact trophozoites has been demonstrated in metabolic flux experiments by measuring the release of radiolabeled CO₂ from [guanidino-¹⁴C]arginine (7). These are all properties consistent with the ADH pathway representing a major metabolic capacity of the protozoan.

We are interested in characterizing the first enzyme in the pathway, arginine deiminase, which catalyzes the irreversible conversion of arginine to citrulline, primarily as a candidate for future drug design. Very few sequences of arginine deiminase are available from genome data bases, and there is very little homology between these published sequences, which prevents the cloning of the arginine deiminase gene from *Giardia* from degenerate oligonucleotides. We have recently reported the purification of arginine deiminase from *Giardia* and the sequence of five tryptic peptides (10). In this paper, we present the first report of the isolation, characterization, and overexpression of the arginine deiminase gene from a eukaryotic organism, and we suggest that it may be an attractive chemotherapeutic target against giardial infection.

* The abbreviations used are: ADH, arginine dihydrolase; PAGE, polyacrylamide gel electrophoresis; nt, nucleotide(s); bp, base pair(s); kb, kilobase(s); PCR, polymerase chain reaction; IPTG, isopropyl-β-D-thiogalactoside; MALDI-TOF, matrix assisted laser desorption-time of flight; CHEF, contour-clamped homogeneous electric field.
Arginine Deiminase Gene from G. intestinalis

EXPERIMENTAL PROCEDURES

Cell Culture Conditions—G. intestinalis trophozoites, Portland I strain, were cultured as described previously (11). Encystation of trophozoites was as described previously (12).

Nucleic Acid Isolation—G. intestinalis genomic DNA was prepared by the method described by Yee and Dennis (13), followed by cesium chloride equilibrium density centrifugation. Total RNA was isolated using TRIzol (Life Technologies, Inc., Grand Island, NY) with 500 mg of genomic DNA.

Expression of the Recombinant Protein—The recombinant KpnI 100 M15(pREP 4) cells (Qiagen) and grown in LB media supplemented with 0.5 mM NaCl, 10 mM imidazole. The histidine-tagged recombinant protein was absorbed onto the column and was washed twice with 10 and 16 ml of start buffer containing 10 and 50 mM imidazole, respectively. The recombinant protein was eluted with 5 ml of elution buffer (20 mM phosphate buffer, pH 7.4, 0.5 mM NaCl, 500 mM imidazole) in 1-ml fractions. Purified recombinant and total cellular protein were quantified using the Bio-Rad protein assay. Recombinant arginine deiminase activity was measured by the colorimetric determination of citrulline formation (9).

Inhibition of the Recombinant Enzyme—Inhibition of recombinant arginine deiminase activity by various arginine analogues was performed, and the results were compared with their previously reported effects on the native enzyme (9). Inhibition studies were in the presence or absence of potential inhibitors at 10 mM concentration with 1 mM arginine, 3.5 μg/ml of protein, 20 mM Hepes, pH 7.0, in the assay.

Nucleic Acid Hybridizations—Trophozoite genomic DNA (10 μg) was digested to completion with either BamHI, EcoRI, HindIII, or PstI, and the resulting segments were separated by electrophoresis on 1% (w/v) agarose gels. The DNA was transferred to Hybond N membrane (Amersham Corp.) and probed overnight at 55 °C with a randomly labeled oligonucleotide ADI 1840 (nts 1840–1859 of the giardial arginine deiminase sequence) and an oligo(dT) primer SSO-10 (CGAGCTTCGTCGACAGGC(T)5) (12). The resulting PCR product was cloned into the pGEM-T vector (Promega, Madison, WI) for sequencing.

Nucleic Acid Hybridizations—Trophozoite genomic DNA (10 μg) was digested to completion with either BamHI, EcoRI, HindIII, or PstI, and the resulting segments were separated by electrophoresis on 1% (w/v) agarose gels. The DNA was transferred to Hybond N membrane (Amersham Corp.) and probed overnight at 55 °C with a randomly labeled (Megaprome, Amersham Corp.) 600-bp PCR product, amplified with oligonucleotides 4 and 5. Total RNA (15 μg) from trophozoites and encysting cells was separated on a 1.3% (w/v) agarose gel containing 0.9% (v/v) formaldehyde. The RNA was transferred to Biotrace blotting membrane (Bio-Rad) and hybridized overnight at 60 °C with a randomly labeled (MegaPrime, Amersham Corp.) 1.5-kb HindIII/PstI fragment from the arginine deiminase gene.

Poly(A)+ mRNA was isolated from 100 μg of total trophozoite RNA using the Dynabeads oligo(dT) Purification System (Dynal). Total RNA and mRNA were similarly separated on a 1% (w/v) agarose containing 7.5% (v/v) formaldehyde. The total RNA and mRNA were transferred onto nylon membrane and probed with the full-length KpnI/BamHI/HindIII fragment.

Chromosomal Location—Giardia chromosomal blots, prepared from chromosome-sized DNA molecules separated by contour-clamped homogeneous electric field (CHEF) gel electrophoresis (14), were generously supplied by Dr. Jacqueline Ucroft (Queensland Institute of Medical Research). Southern hybridization was performed using the full-length BamHI/KpnI fragment of the ADI gene at 60 °C.

Colony Hybridizations—PstI-restricted DNA fragments of approximately 1 kb were excised from an agarose gel, electroeluted, and ligated into pTZ18 vector that had been digested with PstI and dephosphorylated. Transformation was into the Escherichia coli strain JM101. Colonies were transferred to Hybond N membrane and hybridized overnight at 60 °C with the 600-bp ampiclon that had been randomly labeled (MegaPrime, Amersham Corp.). This procedure was repeated with HindIII-restricted fragments of approximately 3.1 kb.

DNA Sequencing and Analysis—The clones chosen from the PstI and HindIII "mini" libraries were subjected to exonuclease III digestion according to the Erase-A-Base kit instructions (Promega). DNA sequencing was with the Sequenase Version 2.0 sequencing kit (Amersham Corp.) or the Pharmacia T7 sequencing kit (Pharmacia, Piscataway, NJ).

The ANGIS (Australian National Genomic Information Service) network was used for computer analysis of sequence data.

Expression of the Recombinant Protein—A 1.8-kb KpnI/BamHI fragment representing the entire arginine deiminase coding sequence was amplified by PCR from Giardia genomic DNA using Pfu polymerase (Boehringer Mannheim). The KpnI/BamHI fragment was subsequently cloned into the pQE-30 expression vector (Qiagen). The recombinant expression shuttle (pQE-30-ADI) was transformed into E. coli M15 (pREP 4) (Qiagen) and grown overnight with 100 μg/ml ampicillin. Induction of expression with IPTG appeared toxic to the E. coli, thus non-inducing conditions were used for protein expression. Cells were grown at 37 °C with shaking for 5.5 h and harvested by centrifugation at 4 °C for 15 min at 2000 × g. The pelleted cells were washed once in phosphate-buffered saline (1.8 mM KH2PO4, 5 mM K2HPO4, pH 7.4, 0.9% (w/v) NaCl) and resuspended in phosphate buffer (20 mM phosphate buffer, pH 7.4, 500 mM NaCl, 10 mM imidazole, 0.1 mM leupeptin). Cell lysates were prepared by sonication on ice at a concentration of 1 g of cells to 5 ml of buffer using a Branson Sonifier 250 (40% duty cycle, output 2–3, 2-min bursts). Cell extracts were centrifuged at 4 °C for 40 min at 27,000 × g to remove particulate material.

Enzyme Purification—Purification of the recombinant protein was performed using a His-trap chelating column (Pharmacia Biotech Inc.) equilibrated with 10 ml of start buffer (20 mM phosphate buffer, pH 7.4, 0.5 mM NaCl, 10 mM imidazole). The histidine-tagged recombinant protein was absorbed onto the column and was washed twice with 10 and 16 ml of start buffer containing 10 and 50 mM imidazole, respectively. The recombinant protein was eluted with 5 ml of elution buffer (20 mM phosphate buffer, pH 7.4, 0.5 mM NaCl, 500 mM imidazole) in 1-ml fractions. Purified recombinant and total cellular protein were quantified using the Bio-Rad protein assay. Recombinant arginine deiminase activity was measured by the colorimetric determination of citrulline formation (9).

Matrix Assisted Laser Desorption/Time of Flight (MALDI-TOF) Mass Spectrometry Analysis—Tryptic digestion of purified recombinant protein was performed using modified sequencing grade trypsin (Boehringer Mannheim). Digestion was carried out in a 1-ml reaction volume containing 400 μg of purified recombinant enzyme, 10 μg of trypsin, 25 μl urea, 0.1 mM (NH4)2CO3, 50 mM glycine at 37 °C for 17.5 h. The peptides were separated from salts using a Sep-Pak C18 column (Waters, Millipore). The salts were first eluted with 4 ml of solvent A (0.1% trifluoroacetic acid in water), and then the peptides were eluted in 2 ml of solvent B (80% trifluoroacetic acid, 90% acetonitrile in water) at a flow rate of 1.1000. The plot was checked for 5% nitro blue tetrazolium chloride in 70% dimethylformamide + 5% 4-bromo-3-chloro-3-indolyl phosphate in dimethylformamide. Native molecular mass of the active recombinant protein was determined by gel filtration using a Smart Chromatography System (Pharmacia).

RESULTS

Amplification of a Fragment of the Arginine Deiminase Gene—Oligonucleotide primers designed from tryptic peptides of the purified arginine deiminase were used in PCR to amplify a portion of the target gene. Since the order of the tryptic peptides in the giardial enzyme was unknown, it was necessary to test all possible combinations of sense oligonucleotides from one peptide with antisense oligonucleotides from the other two peptides in PCR, i.e. six oligonucleotide combinations in all (Table I). Only one oligonucleotide combination gave consistent results in PCR (results not shown). Oligonucleotides 4 and 5 gave the single PCR product of approximately 600 bp. The clone had oligonucleotides 5 and 4 perfectly matched to the 5'- and 3'-ends of the amplicon, respectively. Additionally, an amino sequence that could be a possible match to tryptic peptide 6 (10) was present toward the N-terminal end of the translated PCR amplicon. This 600-bp ampiclon was used in Southern hybridization analysis of genomic DNA, with a unique fragment detected for each restriction digest. The fragment sizes detected were 13.5 kb for BamHI, 6.1 kb for EcoRI, 3.1 kb for HindIII, and 3.2 kb for PstI (results not shown).
Cloning and Sequence Analysis—A mini library from 3.2-kb PstI-restricted fragments was constructed and probed with the 600-bp PCR amplicon. Sequences of two positively hybridizing clones P6/19B and P7/19B, representing the PstI fragment inserted into pTZ18 in both orientations, were determined by overlapping DNA fragments generated by exonuclease III digestion. Sequence analysis revealed an open reading frame from nt 1680 of P6/19B and truncated at the 3'-end of the insert. This open reading frame contained amino acids encoded in the originally amplified PCR fragment and an additional sequence identical to tryptic peptide 17 (10). To obtain the 3'-end of the arginine deiminase gene, a HindIII mini library was screened, and two positively hybridizing clones were identified and designated HE1/19 and HE9/19. Additional sequence

**TABLE I**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Oligonucleotide</th>
<th>Nucleotide sequence</th>
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<tbody>
<tr>
<td>14</td>
<td>1</td>
<td>5'-GTI GA (A/G) GGI GCI CCN GCN TA-3'</td>
</tr>
<tr>
<td>23</td>
<td>3</td>
<td>5'-GCTA IGC IGG NGC NCC (T/C) TA-3'</td>
</tr>
<tr>
<td>27</td>
<td>5</td>
<td>5'-GTTA GA (C/T) GA(T/G) TGG AT (A/C/T) GA (C/T) GT-3'</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>5'-AC (G/A) TC IAT CC (T/C) TG (G/A) TA-3'</td>
</tr>
</tbody>
</table>

Letters in parentheses indicate wobble position nucleotides included in the probe mixtures. I indicates inosine residues. N indicates each of the four nucleotides. Tryptic peptide sequences from purified giardial arginine deiminase were previously reported (10). Sense oligonucleotides are odd-numbered and antisense oligonucleotides are even-numbered.
of the arginine deiminase gene was obtained by “primer walking” on both strands using oligonucleotides designed from known sequence. An amino acid sequence consistent with tryptic peptide 14 (10) was located in this additional sequence. Thus all five tryptic peptides derived from the purified enzyme (10) are present in the deduced amino acid sequence of the open reading frame.

Approximately 3700 bp of sequence was obtained from the PstI- and HindIII-derived clones, of which 1743 bp were an open reading frame encoding arginine deiminase. The deduced amino acid sequence is presented in Fig. 1. Restriction sites for HindIII and PstI and the location of oligonucleotides 4 and 5 that generated the original 600-bp PCR product are indicated. Amino acid sequences consistent with the tryptic peptides from the purified enzyme (10) are shown in bold. The 1743-nt open reading frame encodes a polypeptide of 64,090 Da, which is in agreement with the reported molecular mass of 64,000 Da reading frame encodes a polypeptide of 64,090 Da, which is in close agreement with the predicted size of the transcribed gene and an oligo(dT) in PCR with trophozoite cDNA, polyadenylation of the arginine deiminase transcript was found to occur 10 nts downstream of this heptanucleotide sequence (Fig. 1).

Northern Analysis—Total RNA from trophozoites and encysting cells (6 and 24 h in encysting medium) was subjected to Northern blot hybridization with a 1.5-kb HindIII/PstI fragment of the arginine deiminase gene. A band of approximately 1.8 kb was detected (Fig. 2, panel A). Since no introns have been reported for giardial genes to date, this transcript size is in agreement with the predicted size of the transcribed gene sequence plus a polyadenylation tract. The transcript levels for arginine deiminase were of comparable abundance in trophozoites and in 6- and 24-h encysting cells. Levels of mRNA for an internal control whose transcript levels show no developmental variation2 ascertained equal loading of RNA for each condition.

Chromosomal Location—Chromosome-sized DNA molecules were separated using CHEF gel electrophoresis and subjected to Southern hybridization with the 1.8-kb BamHI/KpnI ADI fragment. The ADI gene was located on chromosome 5 (14) of Giardia (Fig. 3).

Sequence Homology with Other Arginine Deiminases—Sequence information for the arginine deiminase gene is avail-

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able from seven sources to date, all prokaryotic organisms, *Mycoplasma arginini* (18–20), *Mycoplasma hominis* (21), *Mycoplasma orale* (21), *Pseudomonas aeruginosa* (22), *Pseudomonas putida* (GenBank™ accession number U07185), *Halobacterium salinarium* (GenBank™ accession number X80931), and *Clostridium perfringens* (GenBank™ accession number X97684). A ClustalW alignment of these amino acid sequences with the giardial sequence is shown in Fig. 4. The giardial protein is the largest reported to date, with the majority of the additional amino acid sequence occurring as a C-terminal extension compared with the other deiminases. Arginine deiminase from *G. intestinalis* was most similar to the *H. salinarium* enzyme with 26% identity and 50% similarity between the two sequences.

Expression and Purification of Recombinant Arginine Deiminase—The giardial arginine deiminase gene was cloned into the expression vector pQE 30 and transformed in *E. coli* M15[pREP 4] cells. Clones containing the pQE 30-ADI construct were isolated, and the recombinant protein was expressed under non-inducing conditions, since induction with IPTG was toxic to the host cell. The active 6x histidine-tagged recombinant protein was purified in a single step using a His-TrapR (Pharmacia) chelating column. This single step resulted in a purification of 33-fold with 67% of enzyme activity being recovered (Table II). Overall 2 mg of the recombinant arginine

### Table II

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total protein</th>
<th>Specific activity</th>
<th>Purification Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>96</td>
<td>1095</td>
<td></td>
</tr>
<tr>
<td>His-Trap</td>
<td>1.92</td>
<td>36.5</td>
<td>33 67</td>
</tr>
</tbody>
</table>

**FIG. 4.** Alignment of the deduced amino acid sequences of arginine deiminases from *G. intestinalis* (accession number U49236), *M. arginini* (accession number X54141), *M. hominis* (accession number D13314), *P. aeruginosa* (accession number X14694), *P. putida* (accession number U07185), *H. salinarium* (accession number X80931), and *C. perfringens* (accession number X97684) using the ClustalW program. Asterisks indicate identical amino acids, and a single dot indicates a conserved amino acid substitution.

**FIG. 5.** SDS-PAGE and Western blot analysis of purified recombinant arginine deiminase and cell-free extracts. Panel A, lanes 1–3 are molecular mass standards, purified recombinant arginine deiminase, and a cell-free extract of *E. coli* expressing the pQE-3-ADI plasmid, respectively, and stained with Coomassie Brilliant Blue R250. Panel B, lanes 2 and 3 are the same as panel A and probed with nickel-NTA/alkaline phosphatase conjugate, directed specifically against the 6x histidine tag on the recombinant protein.
deiminase could be purified from 96 mg of starting material. To maintain enzyme activity for longer periods the elution buffer was exchanged for 20 mM phosphate buffer, pH 7.4, 0.1 mM EDTA, 10 mM β-mercaptoethanol, 20% glycerol. E. coli containing the expression vector alone had no detectable arginine deiminase activity.

SDS-PAGE analysis indicated the purified recombinant protein had a molecular mass of 66 kDa (Fig. 5). The native molecular mass of the active recombinant enzyme was determined to be 140 kDa by gel filtration, indicating that the active

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**Fig. 6. MALDI-TOF mass spectrometry analysis.** *Panel A,* MALDI-TOF mass spectrum of tryptic peptides of purified recombinant arginine deiminase. The x axis represents the mass charge ratio (m/z), and the y axis represents the relative abundance for each peptide. *Panel B* shows the putative amino acid sequence of the recombinant protein and the tryptic peptides that were observed. Arrows indicate the tryptic peptides for which peaks with the correct mass (± 0.2%) were observed in the spectrum shown in *Panel A.* Broken arrows indicate pairs of tryptic peptides with identical masses. Tryptic peptides that would not be observed in the mass spectrum shown in *Panel A* because their mass is below 750 Da are underlined.
The influence of arginine analogues at a final concentration of 10 mM on the activity of recombinant arginine deiminase with 1 mM arginine at 37 °C over 10 min was studied.

<table>
<thead>
<tr>
<th>Arginine analogue</th>
<th>Enzyme activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Native enzyme</td>
</tr>
<tr>
<td>Alpha amino group blocked or absent</td>
<td>% control</td>
</tr>
<tr>
<td>4-Guanidino butyric acid</td>
<td>96</td>
</tr>
<tr>
<td>Alpha carboxyl group absent</td>
<td>55</td>
</tr>
<tr>
<td>Putrescine</td>
<td>54</td>
</tr>
<tr>
<td>Guanidino group altered or absent</td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>100</td>
</tr>
<tr>
<td>Ornithine</td>
<td>24</td>
</tr>
<tr>
<td>Side chain altered</td>
<td></td>
</tr>
<tr>
<td>Canavanine</td>
<td>29</td>
</tr>
<tr>
<td>Homoarginine</td>
<td>16</td>
</tr>
</tbody>
</table>

*Results were previously reported (9).*

Arginine deiminase is a dimer. Western analysis with a nickel-NTA/alkaline phosphatase conjugate that binds to the histidine tag identified the purified recombinant protein and a single band from an *E. coli* extract expressing the pQE-30-ADI construct (Fig. 5).

Tryptic peptides were analyzed by MALDI-TOF mass spectrometry. The experimental mass spectrum corresponded to the mass profiles predicted for the amino acid sequence data of the recombinant arginine deiminase (Fig. 6). A total of 19 mass peaks that were observed could account for more than 50% of the putative sequence of the recombinant protein.

**Inhibition of the Recombinant Enzyme**—Inhibition of recombinant arginine deiminase activity by various arginine analogues at a concentration of 10 mM in the presence of 1 mM arginine was assessed. The arginine analogue inhibition profile for the recombinant enzyme (Table III) and the native enzyme (9) was comparable.

**Discussion**

*G. intestinalis* as a representative of one of the earliest diverging branches of the eukaryotic tree (5) has retained many prokaryotic features. We have previously demonstrated that the arginine transport system in *Giardia* is supportive of its position as a transition between prokaryotes and eukaryotes, and the metabolism of arginine also reflects the primitive nature of this parasite. Here we report the cloning and expression of small peptides from the arginine deiminase gene. The recombinant enzyme was much more stable than the native enzyme, which loses all activity within 8 h of isolation (10). Further characterization by MALDI-TOF mass spectrometry analysis of a tryptic digest of the purified recombinant arginine deiminase showed a mass spectrum consisting of peaks corresponding to mass-charge ratios of specific tryptic peptides of the recombinant arginine deiminase. The only mass peaks that were observed and could not be accounted for by the tryptic hydrolysis of the recombinant protein were a small peak (m/z 1622.0) and the highest mass peak (m/z 3853.8). The 28 amino acids that were not observed as tryptic peptides may be accounted for by incomplete hydrolysis, by the generation of small peptides (m/z <750) or by losses of polar peptides during desalting. The approach provides a quick and simple method for confirming the sequence of an expressed protein. Although a peptide containing the N-terminal 6x histidine tag was not observed, the presence of the tag was demonstrated by Western analysis (Fig. 5) and by the effectiveness of the one-step purification procedure. Thus it appears that the physical and kinetic properties of the recombinant and native enzymes are comparable.

In addition to being specific for the parasite, it is desirable that a new chemotherapeutic agent has minimal deleterious effects on the human host. For this reason, metabolic pathways and cell components that are unique to a parasite, or significantly different to those of the host, are desirable targets for future drug design. In this paper, we present a potential site for future studies on the regulation and mechanism of arginine deiminase in *Giardia*. Availability of large quantities of recombinant enzyme should pave the way for future studies on the regulation and mechanism of arginine deiminase.
deiminase action in *Giardia*. Furthermore, availability of the recombinant protein will facilitate the pursuit of detailed knowledge of enzyme structure that is required for designing synthetic competitors of this enzyme to combat giardial infection.

REFERENCES

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